TRYPANOSOMES INFECTING GAME AND DOMESTIC STOCK IN THE LUANGWA VALLEY, NORTH EASTERN RHODESIA

BY
ALLAN KINGHORN
AND
WARRINGTON YORKE

(Third interim report of the Luangwa Sleeping Sickness Commission, British South Africa Company)

(Received for publication 22 July, 1912)

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I. INTRODUCTION

In two previous reports (1912), brief references have been made to various trypanosomes found in game and domestic animals in the vicinity of Nawalia, Northern Rhodesia, but as these papers dealt exclusively with the human parasite (*Trypanosoma rhodesiense*, Stephens and Fantham), no details were given concerning the other organisms encountered. It is proposed to deal with these at greater length in the present communication.

Within the confines of the Luangwa Valley a numerous and varied selection of game is found, despite the fact that *Glossina morsitans* is everywhere abundant, but, on the contrary, due doubtless to the presence of these insects, domestic stock is extremely scarce, and in many districts, non-existent. A few goats are occasionally found, but some evidence exists to show that these animals are not so insusceptible to trypanosomiasis, under natural conditions, as is locally supposed. Dogs are very seldom seen, and the natives, themselves, recognise the impossibility of keeping them in the midst of 'fly.' Cattle were found in one village only.

II. METHODS

In our experience, trypanosomes are more readily detected in a buck's blood by the examination of thin, stained smears, than by that of fresh preparations. Except under exceptional circumstances, several hours elapsed after an animal had been shot before its heart reached the laboratory, and this, together with the great heat, had a very deleterious effect on the parasites, destroying their motility, and permitting degenerative changes to occur. In several instances in which fresh preparations, made under these conditions, were examined, as well as blood smears made in the field and afterwards stained, no trypanosomes were found in the fresh blood, whereas they were in the permanent preparations.

In making smears, it was found advisable to cut the animal's throat immediately it had been shot, and to obtain the blood from one of the arteries. It is claimed that such films have the following advantages:—

1. Clean, uncontaminated preparations are obtained.
2. The trypanosomes have had no opportunity of degenerating, and thus stain more sharply.
3. The preparations are permanent.
4. The parasites can be identified more easily in stained, than in fresh preparations. In this particular, the examination of thin films has an obvious advantage over that of thick films.

The preparations were dried in the air, fixed in absolute alcohol for ten to fifteen minutes, and stained with Giemsa.

Owing to the impossibility of obtaining 'clean' sheep and goats, all the inoculations from game were made into monkeys and rats, the amount of blood used varying from 1-10 c.cm. It is recommended that, when possible, sheep and goats be used as well, since they are susceptible to most of the pathogenic trypanosomes, whereas animals such as dogs, monkeys and rats, are not. This is an important consideration when dealing with such parasites as *Tryp. vivax* and *Tryp. nanum.*

III. EXAMINATION OF GAME

A total of 127 head of game, comprising nineteen genera, was examined at Nawalia, and trypanosomes were found by direct examination, by inoculation, or by both methods, in thirty-three. Details are given in Table 1.

Table 1.—Results of examination of game for trypanosomes

Animal	Number examined	Number in which trypanosomes were found in buck's blood	Number inoculations made	Number positive inoculations in which parasites were seen in buck's blood	Number positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculation
1. Elephant	1	0	1	0	0	0
2. Rhinoceros... ...	1	0	1	0	0	0
3. Hippopotamus ...	1	0	0	0	0	0
4. Zebra	5	0	3	0	0	0
5. Roan	8	1	2	0	0	1
6. Wildebeest... ...	2	0	1	0	0	0
7. Kudu	7	3	3	1	1	4
8. Hartebeest ...	6	0	1	0	1	1
9. Waterbuck ...	28	16	14	5	1	17
10. Puku	10	1	6	0	0	1
11. Mpala	29	1	13	1	1	2
12. Bushbuck	9	4	6	1	2	6
13. Bushpig	4	0	1	0	0	0
14. Warthog	9	0	3	0	1	1
15. Lion	2	0	0	0	0	0
16. Hunting Dog ...	1	0	1	0	0	0
17. Giant Rat	1	0	0	0	0	0
18. Genet	2	0	0	0	0	0
19. Squirrel	1	0	0	0	0	0
	127	26	56	8	7	33

It will be seen that parasites were found by direct examination in twenty-six cases, a percentage of 20·4. This is a high figure for single observations, and it is probable that had several preparations from each buck been searched, the percentage of successes would have been much greater. In several instances, only a single trypanosome was found in a film covering the greater part of a slide, and this, after a very careful examination extending over two hours.

A more accurate estimate of the percentage of animals harbouring trypanosomes is afforded by considering only those from which inoculations were made. An analysis of these gives the following figures:—

Number of inoculations made	56
Number of positive inoculations in which parasites were found in buck's blood.....	8
Number of positive inoculations in which no parasites were found in buck's blood.....	7
Number of negative inoculations in which parasites were found in buck's blood......	6
Total number found infected	21

These figures show that at least 37·5 % of the local fauna were infected with trypanosomes. Both *Tryp. vivax* and *Tryp. nanum* have been found in game, and to both these species monkeys and rats are refractory, so that no conclusions can be drawn regarding the presence or absence of these trypanosomes in animals in which parasites were not found in the blood smears. Had sheep and goats been available for inoculation, it is probable that many more buck would have been shown to harbour the two organisms in question. As a conservative estimate, the percentage of game actually infected with trypanosomes in the vicinity of Nawalia might be placed at fifty.

A further point, which is brought out in the Table, is that different species of buck appear to vary widely in their susceptibility. Amongst the commoner species trypanosomes were never found, either by direct examination or by inoculation, in zebra, wildebeest and bushpig, and only rarely in roan, hartebeest, puku, mpala and warthog. Waterbuck, bushbuck and kudu were the species found to be most heavily infected.

Table 2.—Percentages of various species of game found infected with trypanosomes

Animal	Number examined	Percentage harbouring trypanosomes
Bushbuck	9	66·6
Waterbuck	28	60·7
Kudu	7	57·1
Hartebeest	6	16·6
Roan	8	12·5
Warthog	9	11·1
Puku	10	10·0
Mpala	29	6·9

To a certain extent, perhaps, these differences may be accounted for by the habitats affected by the various species of game. Kudu and bushbuck, and waterbuck to a lesser extent, are usually found in thick cover, from which they seldom emerge, and where they are more constantly exposed to the bites of tsetse flies. Mpala, puku, and wildebeest are usually found in open country, frequently remaining for the greater part of the day on wide, bare plains, and here the flies are less noticeable than in the bush. Specific differences in the amount of immunity enjoyed by buck are probably, however, of much greater importance.

In Table 3 are given the species of trypanosomes occurring in each animal in which parasites were found. In compiling the table, information obtained from the result of inoculations, where these were made, has been utilised. This enables a differentiation to be made between such parasites as *Tryp. pecorum* and *Tryp. nanum*, which are, morphologically, indistinguishable. *Trypanosoma vivax* has a characteristic morphology, and can thus be identified in blood smears without difficulty.

As would be expected, double infections are not uncommon amongst game, and several instances of this are recorded in the table.

No data exist as to the ultimate effect of infection on game.

All the animals which were shot appeared to be in perfect condition, and presented no objective signs of disease. Whether or no buck succumb to trypanosomiasis it is impossible to say, but they have increased steadily since rinderpest swept through the country, and so it may be assumed that their tolerance to trypanosomes is very great.

TABLE 3.—Trypanosomes found in game

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Bushbuck 1	Negative	*T. pecorum*	*T. pecorum*
.. 2	*T. pecorum* or *T. nanum*	Negative	*T. nanum*
.. 3	*T. sp.*	*T. sp.*	*T. sp.*
.. 4	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
.. 5	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,, 6	Negative	*T. rhodesiense*	*T. rhodesiense*
Waterbuck 1	*T. pecorum* or *T. nanum*	*T. pecorum*	*T. pecorum*
.. 2	*T. pecorum* or *T. nanum*	*T. pecorum* and *T. rhodesiense*	*T. pecorum* and *T. rhodesiense*
.. 3	*T. pecorum* or *T. nanum* and *T. vivax*	Negative	*T. nanum* and *T. vivax*
.. 4	*T. pecorum* or *T. nanum*	Negative	*T. nanum*
.. 5	Negative	*T. pecorum*	*T. pecorum*
.. 6	*T. vivax*	Negative	*T. vivax*
.. 7	*T. vivax*	Negative	*T. vivax*
.. 8	*T. rhodesiense*	*T. rhodesiense*	*T. rhodesiense*
.. 9	*T. pecorum* or *T. nanum* and *T. vivax*	Negative	*T. nanum* and *T. vivax*
.. 10	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
.. 11	? *T. rhodesiense*	Animal died day after inoculation	? *T. rhodesiense*
.. 12	*T. rhodesiense*	*T. rhodesiense* and *T. pecorum*	*T. rhodesiense* and *T. pecorum*
.. 13	*T. vivax*	No inoculation	*T. vivax*
.. 14	*T. rhodesiense* and *T.vivax*	*T. rhodesiense*	*T. rhodesiense* and *T. vivax*
.. 15	*T. vivax*	No inoculation	*T. vivax*
.. 16	? *T. rhodesiense*, and *T. vivax*	No inoculation	? *T. rhodesiense*, and *T. vivax*
,, 17	? *T. rhodesiense*	No inoculation	? *T. rhodesiense*
Kudu 1	Negative	*T. pecorum*	*T. pecorum*
.. 2	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
.. 3	*T. pecorum* or *T. nanum*	*T. pecorum*	*T. pecorum*
.. 4	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
Roan 1	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
Warthog 1	Negative	*T. rhodesiense*	*T. rhodesiense*
Puku 1	*T. vivax*	No inoculation	*T. vivax*
Mpala 1	Negative	*T. rhodesiense*	*T. rhodesiense*
.. 2	*T. pecorum* or *T. nanum*	*T. pecorum* and *T. rhodesiense*	*T. pecorum* and *T. rhodesiense*
Hartebeest 1	Negative	*T. rhodesiense*	*T. rhodesiense*

IV. EXAMINATION OF DOMESTIC STOCK

The domestic animals examined, and the species of trypanosomes found in them, are given in Table 4.

TABLE 4.—Examination of Domestic Stock for Trypanosomes

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Cow	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,, ...	*T. pecorum* or *T. nanum*	Result not yet known	*T. pecorum* or *T. nanum*
Goat No. 39	*T. vivax*	Negative	*T. vivax*
,, ,, 94	*T. vivax* and *T. nanum* or *T. pecorum*	Negative	*T. vivax* and *T. nanum*
,, ,, 202	*T. pecorum* or *T. nanum*	Negative	*T. nanum*
,, ,, 258	*T. vivax*	Negative	*T. vivax*
Dog	*T. rhodesiense*	*T. rhodesiense*	*T. rhodesiense*
,, ...	*T. pecorum*	*T. pecorum*	*T. pecorum*
,, ...	*T. pecorum*	No inoculation	*T. pecorum*
,, ...	*T. pecorum*	No inoculation	*T. pecorum*
,, ...	*T. sp.* (? *montgomeryi*)	Negative	*T. sp.* (? *montgomeryi*)

The only native village in which cattle were found was Kambwiri's, some forty miles south-east of Nawalia. At present there are only three head, all that are left of a big herd which existed there some four or five years ago. Two of the three appeared to be in good condition when seen, but the headman of the village fully expected to lose them within a few months. The third beast was obviously ill. The cow in which trypanosomes were found, at Fort Jameson, was bred on the Government Farm, and had never been beyond the limits of the township. Tsetse flies have never been seen within some miles of the place, but *Stomoxys sp.* is abundant in the kraals, and, at certain seasons of the year, various species of *Tabanidae* are common.

In several of the villages on the main road from Nawalia to Fort Jameson, a number of goats were found at the end of August, 1911, and again at the beginning of April, 1912, but at the end of that month not a single animal was alive. *Glossina morsitans* was found around all these villages. The four goats mentioned in Table 4 were under observation at Nawalia for a considerable length of time. During this period, parasites were found in the peripheral blood only at rare intervals. Two were rather thin, but not markedly so, and, apart from this, there were no signs of disease. Goat No. 258 was examined at frequent intervals for two months before parasites were first found, while in the others, trypanosomes were seen on the first occasion. Nos. 39 and 258, after having been under observation for nine and four months, respectively, died on the road when the Commission left Nawalia, most probably from being overdriven. The other two are still alive, seven and four months after the diagnosis was made.

The dog in which *Tryp. rhodesiense* was found, came from a village just on the Nyasaland border. The natives said that it had not been out of the village for over a year previously. As the disease runs an extremely acute course in these animals, there can be no doubt that the dog was infected locally.

V. DESCRIPTION OF TRYPANOSOMES

1. *Trypanosoma rhodesiense.*

This parasite has been fully dealt with in the previous reports, and requires, therefore, no further description. It was isolated from four waterbuck, two mpala, one hartebeest, one bushbuck and one warthog—16 % of the game from which inoculations were made—and from one native dog. Parasites resembling *Tryp. rhodesiense* were found in blood films made from three other waterbuck, from which no sub-inoculations were made.

2. *Trypanosoma vivax.*

This organism was found in eight waterbuck, one puku, and three goats.

Morphology

(*a*) In fresh preparations it appears as a club-shaped organism, characterised chiefly by the extraordinary rapidity with which it moves across the field.

(*b*) In stained preparations it is seen to be more or less club-shaped, with a long, free flagellum. The greatest width is posterior to the nucleus, which is situated about the middle of the body. The blepharoplast is large and rounded, and lies close to the posterior extremity of the parasite. The undulating membrane is, as a rule, very feebly developed, or absent.

The mean length of 125 trypanosomes was 23·3μ, the maximum 28·25μ, and the minimum 19·75μ. The greatest width varied between 2 and 4·25μ, average 3·2μ.

Pathogenicity

Inoculations were made into the following animals:—

4 Monkeys	... ...	all remained negative	
1 Rabbit	... ...	,,	,,
2 Rats	... ...	both ,,	,,

Transmission

The vector is probably *Glossina morsitans*. Experiments are now in progress.

3. *Trypanosoma nanum.*

Found in the following animals:—one bushbuck, three waterbuck, and two goats. Possibly it was also present in two other bushbuck, one other waterbuck, two kudu, a roan, and two cattle, but as no sub-inoculations were made, it was impossible to differentiate it from *Trypanosoma pecorum*.

Morphology

(*a*) In fresh preparations it appears as a short, sluggish organism. As a rule, it does not progress.

(*b*) In stained preparations it is found to be short, with a more or less rounded posterior extremity from which the body tapers forwards to the acute anterior end. The nucleus is placed at the centre of the body. The blepharoplast is small, and is situated near the posterior extremity. There is no free flagellum, and the undulating membrane is absent, or, at most, very slightly developed. The protoplasm is in general free from vacuoles and granules.

The mean length of 175 individuals was 14·4μ, the maximum 19μ, and the minimum 10μ. The breadth, at the level of the nucleus, varied from 1 to 2·25μ, the average being 1·5μ.

Pathogenicity

The following animals were inoculated:—

3 Monkeys ... ... all remained negative
1 Rabbit ... ... ,, ,,
3 Rats ... ... ,, ,, ,,

Transmission

The vector has not yet been determined with certainty, but some evidence exists to show that *Glossina morsitans* is the principal one. Experiments are now in progress.

4. *Trypanosoma pecorum.*

Found in the following animals:—one bushbuck, one mpala, four waterbuck, two kudu, three dogs, and one wild rat. It was possibly present in two other bushbuck, a fifth waterbuck, two other kudu, a roan, and two cattle, but as mentioned before, it could not be distinguished from *Trypanosoma nanum*, in the absence of sub-inoculations.

Morphology

(*a*) In fresh preparations, this parasite resembles *Trypanosoma nanum* very closely. It is a short, sluggish organism, showing no degree of translatory power.

(*b*) In stained preparations it appears as a short organism, with an obtuse or rounded posterior extremity from which it tapers to the attenuated anterior end. The nucleus is oval or rounded, situated near the middle of the body. The blepharoplast is small and rounded, and lies close to the posterior extremity. The undulating membrane, if present, is very feebly marked, and there is no free flagellum. The general protoplasm stains uniformly, and is devoid of granules and vacuoles.

The mean length of 400 individuals was 13·6μ, the maximum 19μ, and the minimum 9·5μ. The breadth, at the level of the nucleus, varied from 1 to 2·5μ, the average being 1·5μ.

Pathogenicity

A synopsis of the pathogenicity is given in Table 5.

TABLE 5.—Pathogenicity of *Trypanosoma pecorum*

Animal	No. inoculated	Incubation period days	Duration days
Monkey	9	8-14, average 11	28-225 (still alive)
Rabbit	1	10	215
Guinea-pig ...	1	5	39
Rat	11	3-11, average 5½	6-32
Mouse	2	5	27-75

Transmission

Trypanosoma pecorum was obtained in one of twenty-eight experiments in which freshly-caught *Glossina morsitans* were fed on clean monkeys. While, therefore, this is sufficient to demonstrate the ability of this fly to transmit the parasite, there is a mass of circumstantial evidence which indicates that it is not the only vector, and that possibly it is not the most important one. In the vicinity of Nawalia alone, *Trypanosoma pecorum* was found in 7-9·4 % of the game examined, and in three of five dogs. In addition to these, it was found in one wild rat which had been kept in the laboratory for some weeks in a wide-meshed wire cage. Various species of Tabanids were seen entering the cage, but it is impossible to say whether the rat was infected by one of these. In all, 3,202 freshly-caught *Glossina morsitans* were fed on clean monkeys, and while *Trypanosoma rhodesiense* was obtained in five of the experiments, *Trypanosoma pecorum*, which is of frequent occurrence in game, was only recovered in one.

In a recent number of the Sleeping Sickness Bulletin (1912) the information regarding the rôle of insects, other than tsetse flies, in transmitting trypanosomes is reviewed, and shows that there is an increasing amount of circumstantial evidence that various species of *Tabanidae*, more particularly, may do so. Within the past four years there have been three outbreaks of trypanosomiasis amongst cattle on farms near Fort Jameson, and also on the Government Farm there. Here, tsetse flies may be absolutely excluded. *Stomoxys* and *Lyperosia* are constantly present in the kraals, and during the rains various species of *Tabanus*, *Pangonia*, and *Haematopota* are common. It appears probable, therefore, that some of these species may be important factors in the spread of trypanosomes, but in the absence of definite experiments to determine the point, no conclusions can be drawn.

5. *Trypanosoma sp.*

This parasite, which has not yet been fully worked out, was isolated from one bushbuck.

Morphology

(*a*) In fresh preparations, the trypanosome is seen to be markedly polymorphic, resembling, in this particular, *Trypanosoma rhodesiense*, *Trypanosoma gambiense*, and *Trypanosoma brucei*. Short, sluggishly-moving forms are seen, as well as long, free-flagellated, active ones. As a rule, the short varieties are more or less stationary, while the long ones progress fairly rapidly. The relative number of each of the forms varies widely, from day to day, in any one animal.

(*b*) In stained preparations, every gradation between extremely short, aflagellar forms, indistinguishable from *Tryp. pecorum*, and long, slender, free-flagellated parasites are seen. In general, the trypanosome morphologically closely resembles *Tryp. gambiense* or *Tryp. brucei*, except for the presence of occasional *pecorum*-like forms.

The mean length of 525 individuals was 20μ, the maximum $33{\cdot}25\mu$, and the minimum $10{\cdot}75\mu$. The curve representing the distribution of the various lengths of the trypanosomes, expressed in percentages of the total number measured (525), differs from that of *Tryp. gambiense* in that the apex occurs at 17μ.

Pathogenicity

The reaction of laboratory animals to the trypanosome is given in Table 6.

TABLE 6.—Pathogenicity of *Trypanosoma sp.*

Animal		Incubation period days	Duration, days
Monkey	No. 166 ...	8	94
,,	,, 312 ...	10	Alive after 66
,,	,, 360 ...	7	,, 43
Rabbit	,, 370 ...	6	,, 31
Guinea-pig	,, 371 ...	Negative	
Rat	,, 219 ...	7	104
,,	,, 253 ...	12	Alive after 107
,,	,, 361 ...	10	,, 43

Transmission

Unknown.

6. *Trypanosoma sp.* (?*Montgomeryi.*)

Found in one dog, which was obtained from a village in the hills on the Nyasaland border.

Morphology

(*a*) In fresh preparations, the parasite appears as a broad, stumpy organism, which shows no marked degree of translatory power.

(*b*) In stained preparations, the trypanosome resembles, at first sight, *Tryp. pecorum*, but on closer examination it can be readily distinguished from this parasite by its great breadth. The ratio of the breadth to the length is 1 : 4·8. The posterior extremity is sub-acute or rounded, the anterior attenuated. The greatest width lies at the level of the nucleus, which is situated at the middle of the body, or posterior to it. The blepharoplast is very large and rounded, and is situated near the posterior extremity. Frequently it lies at one edge of the trypanosome, and projects laterally as a small excrescence. The undulating membrane is, as a rule, absent, and when present is simple, and feebly developed. Occasionally, a short, free flagellum, 1-2μ in length, is to be seen, but this is generally absent. The cytoplasm often contains coarse granules and vacuoles. The latter are most commonly seen in the posterior portion of the body, whereas the granules may be scattered generally throughout the protoplasm.

The average length of 200 individuals was 15·88μ, the maximum 20μ, and the minimum 10μ. The breadth, at the level of the nucleus, varied from 1·25 to 6·5μ, the average being 3·29μ.

Pathogenicity

One rat was sub-inoculated from the dog, and had not become infected up to the thirteenth day afterwards, when it was accidentally killed. Unfortunately, the dog died some days previously, so that the strain was lost.

Transmission

Unknown.

Diagnosis

It will be seen that, morphologically, this parasite differs widely from *Tryp. pecorum.* The average length is greater, 15·88μ as compared with 13·6μ. The most characteristic difference, however, is the great breadth of the organism, which is, on an average, 2·2 times that of *Tryp. pecorum*; average breadth 3·29μ as compared with 1·5μ. It appears to resemble most closely the parasite described by Montgomery and Kinghorn (1909) as the Ninamwenda strain, and for which the name *Trypanosoma montgomeryi* was proposed by Laveran. But, in view of the fact that we were able to examine material from one dog only, we cannot regard its identity with this parasite as established.

VI. SUMMARY

1. Trypanosomes are of frequent occurrence in game and domestic stock in the Luangwa Valley. At least 37·5 % of the buck harbour parasites.

2. Six species of trypanosomes were found, viz.: *Tryp. rhodesiense*, *Tryp. vivax*, *Tryp. nanum*, *Tryp. pecorum*, and two others, of which, one was possibly *Tryp. montgomeryi*.

3. *Glossina morsitans*, in nature, transmits two of these trypanosomes, viz.: *Tryp. rhodesiense* and *Tryp. pecorum*, and probably also transmits at least two others, namely, *Tryp. vivax* and *Tryp. nanum*.

4. Circumstantial evidence exists to show that *Tryp. pecorum* may be transmitted by biting insects other than tsetse flies.

NGOA, N. RHODESIA,
May 28, 1912.

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